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Formation of dioxins from triclosan with active chlorine: A potential risk assessment

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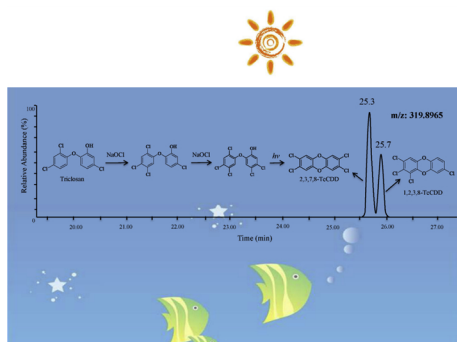
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ABSTRACT

Triclosan, a widely used antimicrobial agent, can increase colitis-associated colon tumorigenesis, and induce liver fibrosis and cancer in mice through mechanisms which may be relevant in humans. In this study, an analytical method using gas chromatography-mass spectrometry (GC-MS) and high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) was developed to measure dioxins and chlorinated derivatives from triclosan in the presence of active chlorine in seawater matrix. Formation yields of dioxins and chlorinated triclosans were assessed at different initial precursor concentrations under dark and UV light irradiation conditions. Results showed that triclosan was rapidly transformed to its chlorinated derivatives, i.e. tetrachlorosans and pentachlorosans, of which the formation yields peaked after 1 h of reaction. UV light was the key factor to promote the formation of dioxins. With the same initial triclosan/active chlorine ratio, the highest yield of dioxins was observed with lower initial concentrations of triclosan under UV irradiation. Five dioxins, including 2,8-DCDD, 1,2,8-TrCDD, 2,3,7-TrCDD, 1,2,3,8-TeCDD, and 2,3,7,8-TeCDD, were identified and quantified. 2,3,7,8-TeCDD, the most toxic dioxin, was firstly reported as the photo-transformation product of triclosan in aquatic solution. Results presented here are useful for a comprehensive understanding of the fate and toxicity of triclosan in contaminated waters.

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1. Introduction

Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol) is a widely used broad-spectrum antimicrobial and antibacterial agent. It is suspected that a large amount of household disinfectants and antiseptics containing triclosan were used after the outbreak of severe acute respiratory syndrome (SARS) in 2003 due to the need to against SARS coronavirus [1]. Being an anti-bacterial and anti-fungal agent, triclosan has also been included in a wide range of pharmaceutical and personal care products since then [2]. This leads to the discharge of a large amount of triclosan in densely populated cities such as Hong Kong, via domestic sewage effluent. Studies have revealed that triclosan would degrade to dioxins when exposed to sunlight in wastewater and seawater [3–7]. It has been detected not only in rivers and lakes in Hong Kong [8–10], but also in the human serum of Hong Kong residents [11], indicating a widespread triclosan exposure in the region.

Recent animal toxicological study showed that triclosan could increase colonic inflammation and associated colon tumorigenesis [12]. Long term exposure of triclosan could also promote liver fibrogenesis and tumorigenesis in mice, and the mechanism of triclosan-induced mouse liver toxicity may be relevant to human [13]. One of the main concerns about the environmental fate of triclosan is the formation of polychlorinated dibenzo-*p*-dioxins through photo-degradation [14,15]. It is commonly known that dioxins are potent multisite carcinogens, and of all the dioxin congeners, 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (2,3,7,8-TeCDD) is the most toxic [16,17]. Therefore, it is reasonable to speculate that the potential adverse human health effects resulting from the long-term exposure to triclosan and its photo-transformation products, e.g. dioxins, could be even more severe than that expected from the triclosan-only exposure.

Some laboratory studies have been carried out to investigate the formation of dioxins from the direct photolysis of triclosan and its chlorinated derivatives under UV and/or sunlight. 1,2,3,8-TeCDD was found as the photo-degradation product of 4,5,6-trichloro-2-(2,4-dichlorophenoxy)phenol under UV radiation of 290–430 nm [7,18]. Kanetoshi et al. [19] detected 2,8-dichlorinated dibenzo-*p*-dioxin (2,8-DCDD), 2,3,7-trichlorinated dibenzo-*p*-dioxin (2,3,7-TrCDD), and 1,2,8-TrCDD from the 20-h UV irradiation of triclosan, 4,5-dichloro-2-(2,4-dichlorophenoxy) phenol, and 5,6-dichloro-2-(2,4-dichlorophenoxy) phenol, respectively. However, 1,2,3,8-TeCDD was not detected from the photolysis of 4,5,6-trichloro-2-(2,4-dichlorophenoxy)phenol [19]. In another study conducted by this group, solid state triclosan and its three above-mentioned chlorinated derivatives were exposed to sunlight on a glass plate for 18 h, and all these above-mentioned dioxins including 1,2,3,8-TeCDD were determined [20]. In the subsequent photo-transformation studies of triclosan, 2,8-DCDD was identified as one of the major transformation products in aqueous environments through a photochemical cyclization reaction [5–7,21–23]. However, none of these studies reported 2,3,7,8-TeCDD as the transformation product of triclosan in the environment.

Among the various oxidants used for water disinfection, chlorine is by far the most widely used. Previous investigations have reported that dioxins and other toxic byproducts, such as chlorinated phenols, chlorinated phenoxy-phenols, and trihalomethanes, were formed from the photo-degradation of triclosan in the presence of free chlorine or through the free-chlorine-mediated oxidation of triclosan [24–28]. These byproducts are 2,4,6-trichlorophenol, 2,4-dichlorophenol, 4,5-dichloro-2-(2,4-dichlorophenoxy) phenol, 5,6-dichloro-2-(2,4-dichlorophenoxy) phenol, chloroform, and so on. Some of these compounds, e.g. chloroform and 2,4,6-trichlorophenol, are classified as potential human carcinogens by the U.S. EPA [29]. In addition, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans are also reported as the products from the electrochemical oxidation of triclosan [30].

Given the well-recognized toxicity and carcinogenicity of dioxins, it is of great environmental and toxicological importance to study the fate

of triclosan and its chlorinated derivatives in the environment. Obviously, more laboratory studies on their aqueous photo-transformation and chlorination chemistry are needed to comprehensively investigate their roles in the formation of dioxins and dioxin-like compounds in aquatic environments, especially in the presence of chlorine. In this study, a sample preparation method using acidic silica gel/acidic alumina/florisil columns was developed to extract the trace amount of dioxin derivatives from samples solutions containing triclosan and its chlorinated derivatives. The enriched dioxin derivatives were then quantitatively analyzed using gas chromatography-mass spectrometry (GC–MS) and high resolution GC-high resolution MS (HRGC-HRMS). Using this developed analytical technique, we are not only able to characterize dioxins and chlorinated triclosans from the transformation of triclosan in the presence of active chlorine in seawater mixture, but also investigated their formation yields and possible formation mechanisms and pathways under both dark and UV irradiation conditions. To the best of our knowledge, it is the first time to identify 2,3,7,8-TeCDD as the aquatic photo-transformation product of triclosan in the presence of active chlorine. This indicates potential adverse human health effects could be caused by the long-term exposure to triclosan and its transformation products in the aquatic environment.

2. Materials and methods

2.1. Chemicals and reagents

Dichloromethane (DCM) and *n*-hexane used in various experiments were of ABSOLV grade (Tedia Company, Inc., Fairfield, Ohio, USA). Silica gel, acidic alumina (Sigma-Aldrich, Inc., Steinheim, Germany), nonane ($\geq 99\%$, Fluka Chemie GmbH, Buchs, Switzerland) were of GC grade. Anhydrous sodium sulphate (granular) was of AR grade. Triclosan was bought from Nippon Kasei Chemical Co., Ltd. (Tokyo, Japan). Other standards including $^{13}\text{C}_{12}$ -labeled triclosan, 2,8-DCDD, 2,3,7-TrCDD, 1,3,6,8-TeCDD, 1,3,7,9-TeCDD, 1,2,4,7-TeCDD, 1,3,7,8-TeCDD, 1,4,7,8-TeCDD, 1,2,3,4-TeCDD, 2,3,7,8-TeCDD, $^{13}\text{C}_{12}$ -labeled 2,3,7,8-TeCDD, 1,2,3,8-TeCDD and 1,2,8,9-TeCDD were purchased from Wellington Laboratories (Guelph, Ontario, Canada).

2.2. Experimental procedure of the photo-transformation of triclosan

The photo-transformation of triclosan was carried out in a UV reactor, which was made by the Workshop of Hong Kong Baptist University. Sixteen 8 W 365 nm UV lamps (Philips, Netherlands) were mounted on the walls of the reactor as the radiation source. The experiment was performed at room temperature (about 25°C) and one electric fan was used to cool down the temperature inside the reactor. Seawater collected from Tai Po Harbor in Hong Kong was filtered and used as the matrix solution, because our previous study showed that it contains very low level of triclosan and no photo-transformation products were detected in it [9]. Given the wide use of sodium hypochlorite (NaOCl) as a disinfectant/bleaching agent in daily life and its presence in environmental waters, NaOCl was chosen as the free chlorine precursor in this experiment. Free chlorine stock solutions were prepared using purified grade NaOCl (Acros Organics, New Jersey, USA). The concentration of total active chlorine in the sample solution is the sum of $[\text{HOCl}]$ and $[\text{OCl}^-]$, and it was measured by the DPD/FAS titrimetric method. Total triclosan includes triclosan and phenolate-triclosan. Given the pKa values of HClO and triclosan are 7.5 and 7.9 respectively, and the pH of seawater matrix was between 7.5 and 8.4, the predominant active chlorine and triclosan species got involved in the experimental system were HClO and phenolate-triclosan.

NaOCl solution and triclosan (dissolved in methanol) were added into the 500 mL seawater matrix and homogenized using a stirring magnetic bar. Initial concentrations of triclosan and active chlorine in the samples were set at 0.2 and 2.0 $\mu\text{g mL}^{-1}$ and 2.0 and 20 $\mu\text{g mL}^{-1}$, respectively. The concentrations of triclosan were within those detected

in the aquatic environment [31]. The percentage of methanol was kept below 0.1% of the sample volume in all experiments. After triclosan reacted for 0 min, 60 min, 120 min, 240 min, and 960 min under UV (365 nm) radiation and 0 min, 60 min, 120 min, 240 min, 960 min, 2 days, 8 days, 32 days, and 64 days in dark, the excess of chlorine was removed by the addition of sodium thiosulphate. Under dark condition, the glass bottles were covered by aluminum foil and securely stopped to avoid sample contamination. After the reaction was quenched by adding an excessive amount of sodium thiosulphate, sample solutions were adjusted to pH 2.5 and pretreated by liquid-liquid extraction and acidic silica/acidic alumina/florisil column clean up procedure described below. The detailed experimental conditions under both dark and UV irradiation conditions are listed in Tables 1 and 2.

2.3. Sample pretreatment

The sample was first extracted with 100 mL of DCM by vigorously shaking the reaction bottles for 5 min, and the organic portion was collected. The extraction was repeated for three times. The combined organic phase was dried using anhydrous sodium sulphate and then evaporated to dryness under a gentle pure N₂ flow. The residue was re-dissolved in 15 mL of hexane, and the hexane solution was then concentrated to 1 mL. An aliquot of 10 µL was taken out and diluted to 1 mL with nonane. ¹³C₁₂-labeled triclosan with the final concentration of 50 ng·mL⁻¹ was added as the internal standard prior to GC–MS analysis.

For the HRGC–HRMS analysis of dioxins, samples were further cleaned up by consecutive acidic silica gel/acidic alumina/florisil columns to remove triclosan, its chlorinated derivatives and other impurities. An acidic silica gel column (upper column) and an acidic alumina column (lower column) were prepared by packing 8 mL of acidic silica gel and 8 mL of acidic alumina into two glass chromatographic columns (1.0-cm inside diameter, 15-cm length), respectively. The two columns were connected in series and rinsed with 15 mL of hexane before usage. Samples re-dissolved in 15 mL of hexane were loaded onto the SPE columns. The sample vial was rinsed with 7 mL of hexane, which was also loaded onto the columns. After adding three portions of 2.5 mL of hexane directly onto the columns, the upper acidic silica gel column was run dry and taken away. The lower acidic alumina column was further washed with four portions of 2.5 mL of DCM/

hexane (6%, v/v) mixture. The reaction products were then eluted with 16 mL of DCM/hexane (60%, v/v) mixture and dried by the pure N₂ flow. The dried residue was re-dissolved in three portions of 2.5 mL of hexane, loaded onto an 8 mL florisil column, and then eluted with 6.0 mL of hexane and 8.0 mL of DCM sequentially. The analytes were in the DCM fraction. After blown dry, the sample was reconstituted in 100 µL of nonane. ¹³C₁₂-labeled 2,3,7,8-TeCDD with the final concentration of 50 ng·mL⁻¹ was added as the internal standard prior to HRGC–HRMS analysis.

2.4. GC–MS analysis

A GC–MS method was developed for the analysis of triclosan and its chlorinated products. Samples were analyzed in both selective ion monitoring (SIM) mode and full scan mode. In this project, SIM mode was used for quantification and “Acquire Scan and SIM data mode” was used for qualitative analysis. An Agilent 6890 N gas chromatography equipped with a split/splitless injector and a HP-5 MS capillary column (30 m × 0.25 mm, 0.25 µm film thicknesses) coupled to Agilent 5975 mass spectrometer was used. One microliter of the sample extract was injected in splitless mode with injector temperature of 250 °C, with helium as the carrier gas. The GC column was held at 100 °C for 2 min, followed by an increase of 3.0 °C/min to 220 °C and held for 10 min. The GC–MSD transfer line temperature, source temperature, and electron ionization (EI) voltage were set at 280 °C, 230 °C, and 70 eV, respectively. Triclosan and its chlorinated products were identified and quantified by comparing the retention time and mass spectrum to those of the authentic standards.

2.5. HRGC–HRMS analysis

A HRGC–HRMS method was developed to determine dioxins as the transformation products of triclosan. An Agilent 6890 series GC equipped with a DB-5MS (J&W Scientific, CA) fused-silica capillary column (60 m × 0.25 mm, 0.25 µm film thickness), and coupled to an Autospec Ultima mass spectrometer (Micromass, Manchester, U.K.) was used. The GC conditions were optimized to achieve the best separation capacity. The initial oven temperature was held at 170 °C for 1.5 min, followed by an increase of 20 °C/min to 220 °C and held for 5 min. It was then increased to 240 °C at 1.0 °C/min, held at 240 °C for 10 min,

Table 1

Experimental conditions and concentrations of triclosan and dioxins in the transformation study of triclosan in seawater matrix in the presence of active chlorine.

Triclosan conc. ^a (µg/mL)	Active chlorine conc. (µg/mL)	Condition of light	Duration	Results of sample analysis (n = 3)					
				Triclosan Conc. (ng/mL)	2,8-DCDD (pg/mL)	1,2,8-TrCDD (pg/mL)	2,3,7-TrCDD (pg/mL)	2,3,7,8-TeCDD (pg/mL)	1,2,3,8-TeCDD (pg/mL)
0.2	2.0	Dark ^b	0 min	200	Trace	ND ^h	ND	ND	ND
	2.0	Dark	60 min	83.6 ± 13.2	Trace	ND	ND	ND	ND
	2.0	Dark	120 min	44.7 ± 5.7	Trace	ND	ND	ND	ND
	2.0	Dark	240 min	27.3 ± 2.6	2.2 ± 0.2	ND	ND	ND	ND
	2.0	Dark	960 min	3.10 ± 0.4	6.3 ± 0.6	ND	ND	ND	ND
	2.0	Dark	2days	1.70 ± 0.2	8.6 ± 1.4	3.3 ± 0.2	2.4 ± 0.4	ND	ND
	2.0	Dark	8 days	0.62 ± 0.05	9.3 ± 1.8	6.8 ± 1.2	4.2 ± 0.3	ND	ND
	2.0	Dark	32 days	0.38 ± 0.02	13.5 ± 1.4	8.9 ± 0.8	7.8 ± 1.2	ND	3.3 ± 0.2
	2.0	Dark	64 days	0.13 ± 0.01	21.4 ± 0.6	15.2 ± 1.8	13.5 ± 1.5	ND	12.2 ± 2.3
	2.0	365nm	0 min	200	Trace	ND	ND	ND	ND
	2.0	365nm	60 min	23.4 ± 5.6	177.1 ± 12.5	358.6 ± 43.2	287.9 ± 18.7	ND	ND
	2.0	365nm	120 min	17.3 ± 3.4	274.7 ± 23.6	469.3 ± 23.6	382.6 ± 23.5	2.3 ± 0.2	4.5 ± 0.9
2.0	2.0	365nm	240 min	3.2 ± 0.8	124.8 ± 11.3	541.8 ± 56.4	693.5 ± 45.7	7.6 ± 1.5	10.5 ± 2.4
	2.0	365nm	960 min	1.4 ± 0.2	106.3 ± 0.9	664.5 ± 78.3	1404 ± 134	15.3 ± 3.1	21.3 ± 4.6
	20	365nm	0 min	2000	8.9 ± 1.2	ND	ND	ND	ND
	20	365nm	60 min	183.1 ± 30.2	832.4 ± 98.3	1623 ± 45.6	1312 ± 98.3	30.5 ± 3.2	23.5 ± 4.2
	20	365nm	120 min	107.5 ± 12.5	1271.3 ± 87.6	2226 ± 129	2146 ± 340	61.4 ± 5.6	46.5 ± 8.3
	20	365nm	240 min	20.3 ± 3.3	550 ± 43.5	2515 ± 231	3205 ± 357	82.7 ± 9.7	63.1 ± 11.2
	20	365nm	960 min	7.5 ± 0.9	480 ± 67.3	3038 ± 187	6489 ± 569	101 ± 13.3	76.2 ± 6.5

^a Total volume is 500 mL. ^b Glass bottles were wrapped with aluminum foil.

Table 2

Experimental conditions and concentrations of chlorinated triclosans in the transformation study of triclosan in seawater matrix in the presence of active chlorine.

Triclosan conc. ^a ($\mu\text{g/mL}$)	Active chlorine conc. ($\mu\text{g/mL}$)	Condition of light	Duration	Results of sample analysis (n = 3)				
				Tetracosan1 (pg/mL)	Tetracosan2 (ng/mL)	Tetracosan3 (ng/mL)	Pentacosan1 (pg/mL)	Pentacosan2 (ng/mL)
0.2	2.0	Dark ^b	0 min	0	0	0	0	0
	2.0	Dark	60 min	12.0 \pm 0.8	14.3 \pm 1.1	53.6 \pm 3.2	58.0 \pm 3.5	69.2 \pm 3.9
	2.0	Dark	120 min	43.0 \pm 3.7	16.3 \pm 1.8	38.3 \pm 2.0	69.0 \pm 4.3	78.8 \pm 6.3
	2.0	Dark	240 min	32.0 \pm 2.2	10.9 \pm 0.6	28.0 \pm 2.9	65.0 \pm 5.6	67.0 \pm 6.6
	2.0	Dark	960 min	8.0 \pm 1.1	8.5 \pm 0.5	25.3 \pm 1.7	30.0 \pm 2.6	29.8 \pm 2.8
	2.0	Dark	2 days	10.0 \pm 0.9	9.3 \pm 0.6	13.5 \pm 1.0	12.0 \pm 0.9	20.7 \pm 1.8
	2.0	Dark	8 days	5.0 \pm 0.6	6.7 \pm 0.5	9.6 \pm 1.1	8.0 \pm 0.3	15.6 \pm 1.3
	2.0	Dark	32 days	3.0 \pm 0.4	3.2 \pm 0.2	7.3 \pm 0.3	15.0 \pm 0.8	16.5 \pm 1.9
	2.0	Dark	64 days	4.0 \pm 0.5	1.6 \pm 0.2	4.6 \pm 0.4	6.0 \pm 0.6	12.4 \pm 0.8
0.2	2.0	365 nm	0 min	0	0	0	0	0
	2.0	365 nm	60 min	25.0 \pm 3.2	20.7 \pm 1.6	54.2 \pm 3.2	18.0 \pm 1.2	28.2 \pm 2.1
	2.0	365 nm	120 min	46.0 \pm 4.0	15.6 \pm 1.8	46.2 \pm 2.8	22.0 \pm 1.9	32.2 \pm 3.6
	2.0	365 nm	240 min	32.0 \pm 2.8	9.7 \pm 0.9	45.1 \pm 3.8	21.0 \pm 1.5	28.6 \pm 1.8
	2.0	365 nm	960 min	12.0 \pm 0.6	2.0 \pm 0.1	26.7 \pm 1.9	8.0 \pm 0.5	12.9 \pm 1.6
2.0	20	365 nm	0 min	0	0	0	0	0
	20	365 nm	60 min	380.0 \pm 27.0	198.3 \pm 22.3	468.3 \pm 25.0	340.0 \pm 23.0	304.5 \pm 28.0
	20	365 nm	120 min	420.0 \pm 25.3	165.2 \pm 18.7	410.9 \pm 30.2	460.0 \pm 34.5	365.3 \pm 29.3
	20	365 nm	240 min	270.0 \pm 18.2	100.6 \pm 7.8	320.4 \pm 19.7	350.0 \pm 28.0	298.6 \pm 22.1
	20	365 nm	960 min	130.0 \pm 5.6	56.7 \pm 1.9	210.5 \pm 16.8	230.0 \pm 8.6	130.5 \pm 10.5

^a Total volume is 500 mL. ^b Glass bottles were wrapped with aluminum foil.

and finally to 300 °C at 5 °C /min and held for 9 min. EI was used and operated in the SIM mode at 10,000 resolving power (5% valley definition). The two most abundant ions in the $[M]^+$ cluster were monitored at a 50 ms dwell time and a delay time of 10 ms. Dioxins were identified and quantified by comparing the retention time and mass spectrum to those of the authentic standards.

2.6. Quality control/Quality assurance

Six-point calibration curves for triclosan, 2,8-DCDD, 2,3,7-TrCDD, 2,3,7,8-TeCDD and 1,2,3,8-TeCDD were constructed with concentrations ranging from 2 ng·mL⁻¹ to 1000 ng·mL⁻¹. Good linearity was achieved over the whole concentration range with $R^2 > 0.999$. Quantifications of 2,8-DCDD, 2,3,7-TrCDD, and 1,2,3,8-TeCDD were performed using external standard method, and the isotope dilution method was used for triclosan and 2,3,7,8-TeCDD. The ¹³C₁₂-labeled triclosan and ¹³C₁₂-labeled 2,3,7,8-TeCDD were added into the standards with the final concentration of 50 ng·mL⁻¹.

Recovery tests were carried out at three concentrations, which were 2 pg·mL⁻¹, 20 pg·mL⁻¹ and 200 pg·mL⁻¹, respectively (Table S1). Triclosan and dioxin standards were spiked into 500 mL of filtered seawater, and pretreated and analyzed following the procedures

described above. Triplicate spiked samples were analyzed for each concentration level. Recoveries of all the analyzed compounds were within the range of 76.2–116.9 %. The intra-day and inter-day precisions were higher than 84.3% and 82.8%, respectively. The limit of detection (LOD) was defined as the concentration of the analyte required to produce a signal with amplitude of at least 3 times of the baseline noise ($S/N \geq 3$). The LODs of triclosan and dioxins were found to be within the range of 0.44–1.16 pg·mL⁻¹ for GC–MS analysis and 0.032 to 0.11 pg·mL⁻¹ for HRGC–HRMS (Table S1), which were well below the levels of these compounds observed in the water samples.

3. Results and discussion

3.1. Identification of triclosan degradation products by GC–MS and HRGC–HRMS

The mixture of standards, including 2,8-DCDD, 2,3,7-TrCDD, 1,3,6,8-TeCDD, 1,3,7,9-TeCDD, 1,2,4,7-TeCDD, 1,3,7,8-TeCDD, 1,4,7,8-TeCDD, 1,2,3,4-TeCDD, 2,3,7,8-TeCDD, 1,2,3,8-TeCDD and 1,2,8,9-TeCDD, were well separated under the GC–MS condition described in section 2.4 (Table S2). The photo-degradation products of triclosan in seawater were first treated by liquid-liquid extraction and

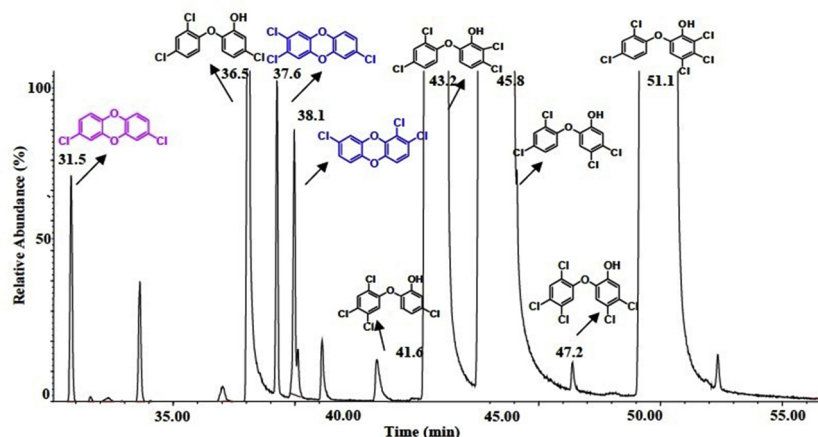


Fig. 1. Full scan GC–MS chromatogram of the photo-transformation products of triclosan at 2.0 $\mu\text{g mL}^{-1}$ in seawater matrix (UV irradiation for 960 min).

analyzed by GC–MS in full scan mode. As shown in Fig. 1, many products were detected in the triclosan photo-transformation experiment, including several dioxins and chlorinated triclosan derivatives. Three tetraclosans, i.e. 5-chloro-2-(2,4,5-trichlorophenoxy)phenol (tetraclosan 1), 5,6-dichloro-2-(2,4-dichlorophenoxy)phenol (tetraclosan 2), and 4,5-dichloro-2-(2,4-dichlorophenoxy)phenol (tetraclosan 3), and two pentaclosans, i.e. 4,5-dichloro-2-(2,4,5-trichlorophenoxy)phenol (pentaclosan 1) and 4,5,6-trichloro-2-(2,4-dichlorophenoxy)phenol (pentaclosan 2) were identified by comparing their mass spectra with the previously reported data [19,20,25,29] and their retention times relative to other known compounds in the GC chromatograms. Several dioxins, such as 2,8-DCDD, 2,3,7-TrCDD, 1,2,8-TrCDD, were also detected by GC–MS. However, for 2,3,7,8-TeCDD (43.4 min) and 1,2,3,8-TeCDD (43.8 min), their retention times were close to that of tetraclosan 2 (43.2 min). Since the concentration of tetraclosan 2 was much higher than these two TeCDDs, they were buried underneath the huge peak of tetraclosan 2 and could not be seen in Fig. 1. Therefore, further cleanup of the sample using the tandem acid silica gel/acidic alumina/florisil SPE columns was performed to remove triclosan and its chlorinated derivatives. The dioxin products were then identified and quantified using both GC–MS and HRGC–HRMS.

The extracted ion chromatography (EIC) of dioxin products in the cleaned samples and their corresponding mass spectra in GC–MS are presented in Figs. S1 and S2. The compound eluted at 31.5 min with a molecular ion at m/z 252 and major fragment ions at m/z 189 ($[M-CO-Cl]^+$) and 126 ($[M-2CO-2Cl]^+$) was identified as 2,8-DCDD, by comparing its retention time and mass spectrum with the authentic standard (Figs. S1A and S2A). The two compounds eluted at 37.6 and 38.1 min had similar mass spectra, i.e. quasi-molecular ion at m/z 286 and major fragmentation ions at m/z 223 ($[M-CO-Cl]^+$) and 160 ($[M-2CO-2Cl]^+$). The former was determined as 2,3,7-TrCDD by comparing to the authentic standard and the latter (Figs. S1B and S2B),

was identified as 1,2,8-TrCDD, which was also reported by Kanetoshi et al. [19,20]. The two tetrachlorodibenzo-*p*-dioxins, 2,3,7,8- and 1,2,3,8-TeCDDs, were clearly observed in the cleaned sample, with their retention times and mass spectra well matched those of the standards (Figs. S1C and S2C).

The EIC of dioxin products in spiked seawater sample using HRGC–HRMS is shown in Fig. 2. Compounds eluted at 12.6, 16.5, 17.8, 25.3, and 25.7 min showed characteristic molecular ion at m/z 251.9743, 285.9354 and 319.8965 (Figs. 2A, 2B and 2C), which well matched those of 2,8-DCDD, 2,3,7-TrCDD, 1,2,8-TrCDD, 2,3,7,8-TeCDD, and 1,2,3,8-TeCDD listed in Table S2. As shown in Fig. 2D, molecular ions at m/z 321.8936 were also observed at 25.3 and 25.7 min, which were from the Cl^{37} -substituted isomers of TeCDD. This doubly confirmed the presence of 2,3,7,8-TeCDD and 1,2,3,8-TeCDD in the sample. Our finding further proved that dioxins could be photo-chemically transformed from triclosan in the presence of free chlorine in seawater matrix. Most importantly, the most toxic dioxin, 2,3,7,8-TeCDD was also determined as the photo-transformation product of triclosan.

3.2. Transformation of triclosan in the presence of active chlorine under dark condition

In the presence of $2.0 \mu\text{g mL}^{-1}$ of active chlorine in dark, triclosan rapidly decayed and its half lifetime was about 30 min (Table 1). The chlorinated triclosans were rapidly formed, and the concentrations of tetraclosan 2, tetraclosan 3 and pentaclosan 2 reached their maxima within 120 min (Table 2). This indicated that triclosan could be easily chlorinated to form tetraclosans and pentaclosans, due to the high oxidability of active chlorine.

Under dark condition, a trace amount of DCDD was detected at the beginning of the reaction. Its concentration was only $2.2 \pm 0.2 \text{ pg mL}^{-1}$ after 240 min, and kept increasing to $21.4 \pm 0.6 \text{ pg mL}^{-1}$ after the system

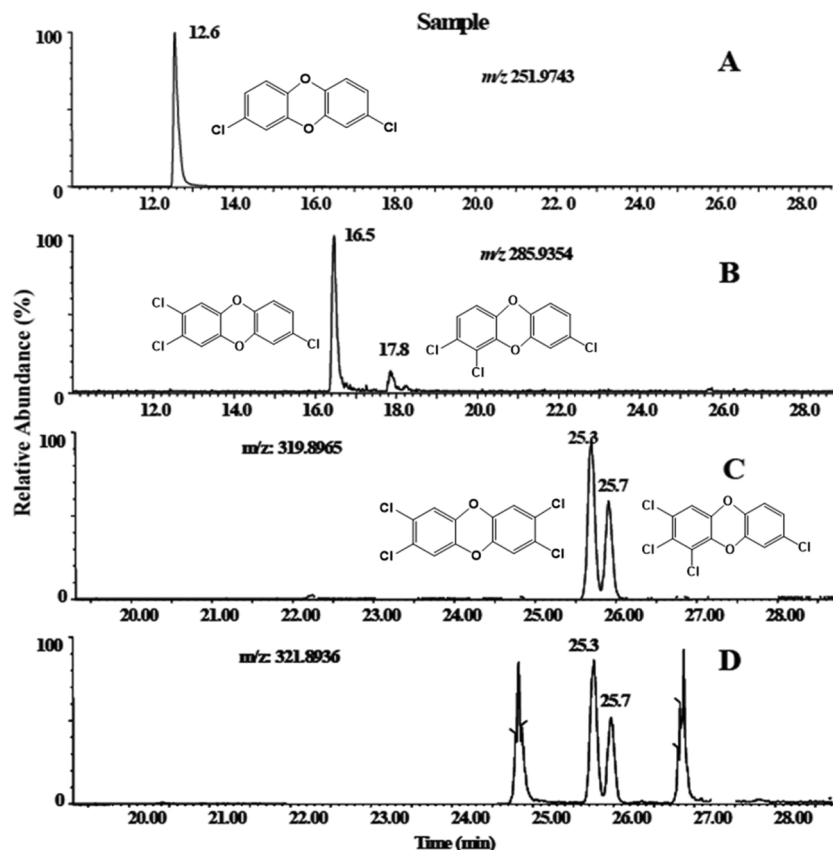


Fig. 2. Extract ion chromatogram (EIC) of m/z 251.9743 (A), 285.9354 (B), 319.8965 (C) and 321.8936 (D) of the photo-transformation products of triclosan in seawater in the presence of active chlorine from the HRGC–HRMS analysis.

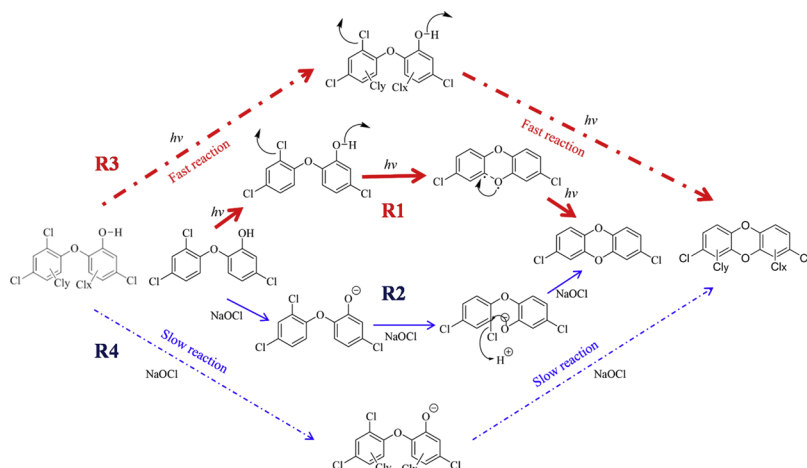


Fig. 3. Proposed formation pathways of dioxins from triclosan and chlorinated triclosan derivatives. Route 1 (R1) and R3, fast photo-transformation under UV irradiation; R2 and R4, slow cyclization reaction under weak base (NaOCl) condition in dark.

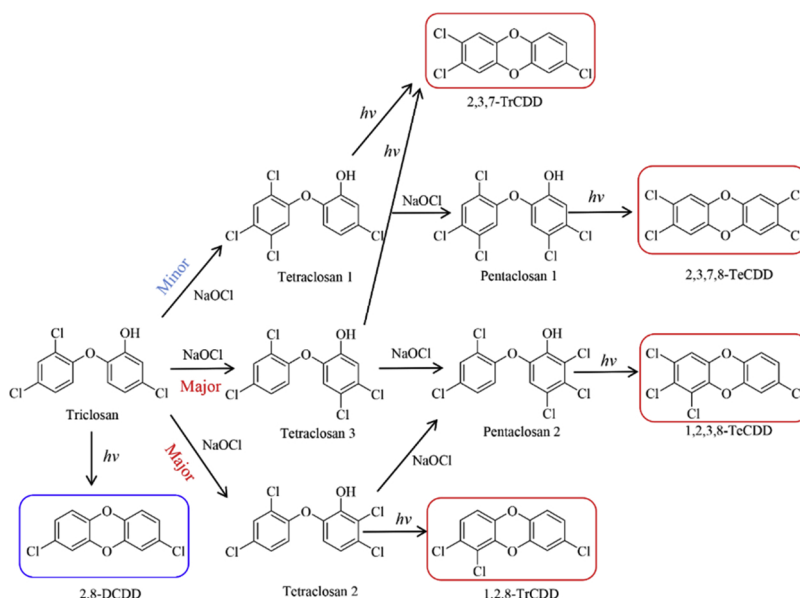


Fig. 4. Proposed transformation pathways of triclosan in the presence of active chlorine.

reacted for 64 days. The 1,2,8-TrCDD and 2,3,7-TrCDD became detectable after triclosan reacted for two days in dark and 1,2,3,8-TeCDD started showing up after 32 days. However, 2,3,7,8-TeCDD was undetectable throughout the dark reaction (Table 1). As mentioned earlier, dioxins were often considered as the photo-transformation products of triclosan. Our finding suggested that under weak base (NaOCl) condition, triclosan could be transformed to dioxins in the presence of excessive active chlorine even in dark. However, their formation rates were rather slow and the formation yields of dioxins decreased with increasing number of chlorine substitutes. As shown in Fig. 3, 2,8-DCDD could be formed through a slow cyclization reaction of triclosan under this condition. Contrary to dioxins, much higher levels of chlorinated triclosan derivatives were observed in the system. Almost all of them reached their highest concentrations at 120 min, and gradually decayed as reaction continued for 64 days (Table 2). Several studies have reported the production of tetracosan 2 and 3 and pentacosan 2 from aquatic triclosan chlorination. These three chlorinated triclosan derivatives were proposed to be formed via bimolecular electrophilic substitution of triclosan and their formation was kinetically favorable [24,25]. Results shown in Table 2 confirmed that these three chlorinated triclosans were the major degradation products of triclosan in dark in the presence of free chlorines. Besides, small amounts of tetracosan 1 and pentacosan 1

were also detected and the formation pathways of all measured chlorinated triclosans were proposed in Fig. 4 [3,32]. Similar to triclosan, these chlorinated derivatives may also undergo a slow cyclization reaction in dark, which led to the formation of TrCDD and TeCDD in the system (Fig. 3).

3.3. Transformation of triclosan in the presence of active chlorine under light condition

In the simulated photolysis experiments of triclosan, UV light at 365 nm was chosen as the radiation source given that triclosan has a high light absorption in the near UV region of solar spectrum. Triclosan decayed much faster under UV radiation than in the dark, and ~90% of it reacted after 60 min of radiation (Table 1). With the same initial concentrations of triclosan and free chlorines, much more dioxins were formed under UV radiation and all of them, including 2,3,7,8-TeCDD were detected as the photo-transformation products of triclosan, given enough reaction time. Among the measured dioxin products, TrCDDs were the most abundant, followed by DCDD and TeCDDs. Concentrations of almost all dioxins increased with increasing reaction time, except for 2,8-DCDD, which peaked after 120 min of radiation and then

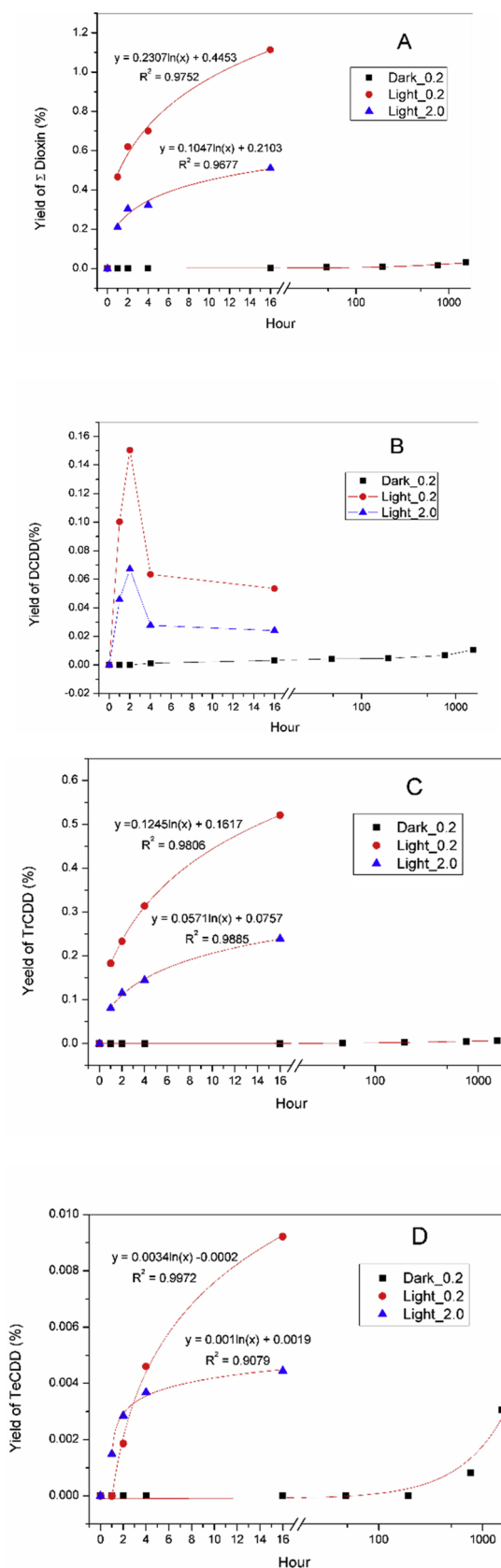


Fig. 5. Formation yields of total dioxins (A), DCDD (B), TrCDDs (C), and TeCDD (D) from the transformation of triclosan at different initial concentrations of triclosan and active chlorine under both light and dark conditions.

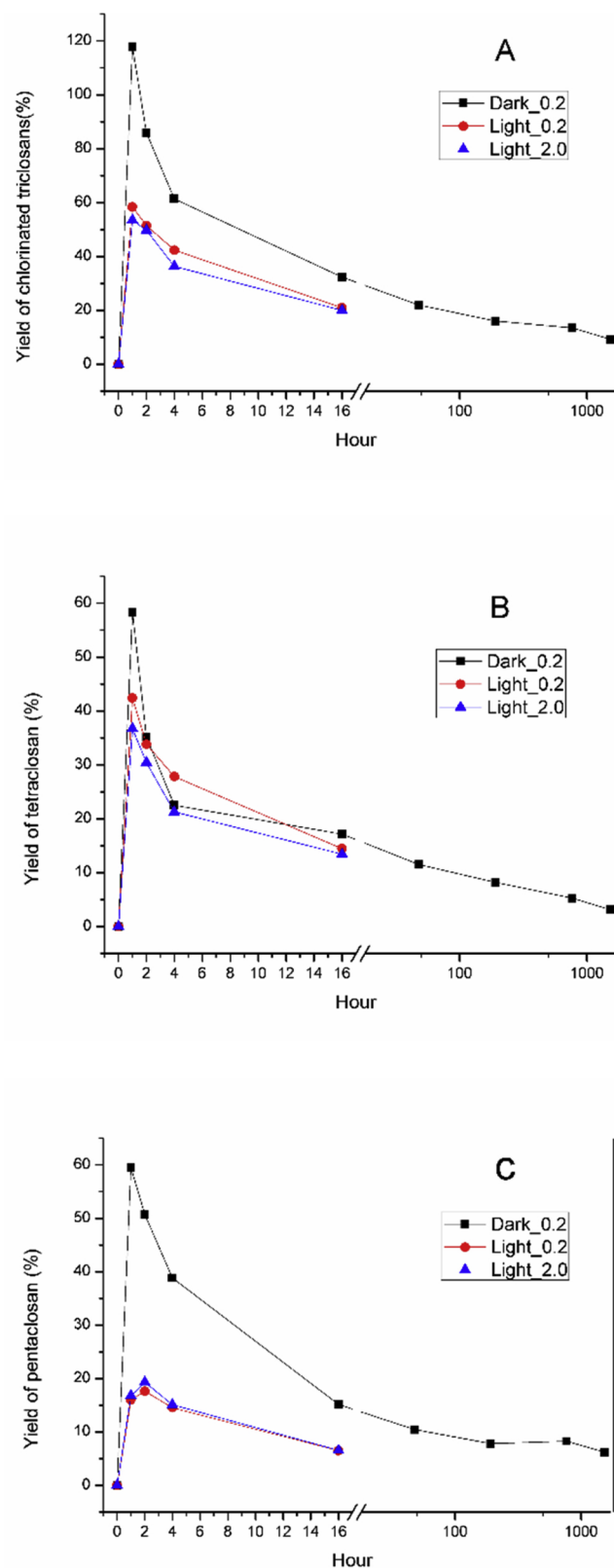


Fig. 6. Formation yields of total chlorinated triclosan derivatives (A), tetrachloros (B), and pentachloros (C) from the transformation of triclosan at different initial concentrations of triclosan and active chlorine under both light and dark conditions.

gradually decreased as reaction continued for 16 h. This demonstrated that 2,8-DCDD was the first generation product from the photolysis of triclosan and it would further degrade to form the higher generation products. One of the possibility was 2,8-DCDD could generate a biradical intermediate upon photochemical excitation, which led to the production of dihydroxy dichlorinated biphenyls and lower chlorinated phenoxyphenols [33]. However, they were not measured in the experiments due to the lack of authentic standards.

3.4. Formation yields and mechanisms of transformation products

In this paper, the formation yield of the transformation products from triclosan was defined as:

$$Y_A = \frac{[A]}{\Delta \text{triclosan}} \quad (1)$$

where $[A]$ is the concentration of the transformation product A (ng/mL), and $\Delta \text{triclosan}$ is the concentration of reacted triclosan (ng/mL). As shown in Fig. 5A, the formation yield of total dioxins under UV radiation was substantially higher than that under dark condition. This confirmed that light was the dominant factor to promote the production of dioxins from triclosan. It is interesting to note that with the same initial triclosan/active chlorine ratio, a higher yield of dioxins was observed in the experiment with lower concentrations of triclosan and free chlorines. Given that the chlorination reactions of triclosan were kinetically favorable, this phenomenon was probably due to the higher possibility for triclosan to undergo chlorination, instead of photolysis when a higher level of active chlorines was present in the system (Fig. 4) [34].

As discussed above, 2,8-DCDD would further degrade through photolysis. We also proposed that 2,3,7,8-TeCDD and 1,2,3,8-TeCDD were the 3rd generation products from the transformation of triclosan through the direct photolysis of pentaclosan 1 and 2, respectively (Fig. 4) [33]. Therefore, it was not surprising to observe that TrCDD had the highest formation yield of all three types of dioxins and TeCDD had the lowest. It was worth noting that the formation yield of pentaclosan 2 was about a thousand times higher than that of pentaclosan 1 under the same experimental conditions (Table 2). Similarly, a much lower yield of tetraclosan 1 was observed than those of tetraclosan 2 and 3. This indicated that the pathway to form 2,3,7,8-TeCDD was minor, compared with those pathways to form TrCDD and 1,2,3,8-TeCDD. However, no significant difference was observed between the yields of 1,2,3,8-TeCDD and 2,3,7,8-TeCDD (Table 1). This indicated that another photo-transformation pathway might exist for 2,3,7,8-TeCDD. Overall, the formation yields of total dioxins were rather low, which was below 1.2% under the tested conditions. As a result, a logarithmic function between the yield of dioxins and reaction time was observed (Figs. 3A, 3C and 3D).

The formation yields of all chlorinated triclosans reached their maximum values after 60 min of reaction and then gradually decayed as reaction continued (Fig. 6). As shown in Fig. 6B, the formation yields of tetraclosans were about the same under dark and light conditions, which indicated that light was not the key factor to promote the formation nor transformation of these compounds (Fig. 4). However, for pentaclosans, their total formation yield was much lower under UV irradiation than that in dark. This indicated that photolysis was an important pathway for the decay of pentaclosans, which might be more significant than the further chlorination of these compounds. This is because pentaclosans possess higher pKa values than that of triclosan (pKa = 7.9–8.1) [35,36]. In the weak basic solution, a smaller fraction of them was in the phenolate forms, and therefore less reacted with active chlorines to get further chlorinated. Besides TeCDDs, other photo-transformation products of pentaclosans could be dihydroxy polychlorinated biphenyls and lower chlorinated phenoxyphenols [33], although they were not monitored in our study. It was interesting to note that the formation yields of tetrachlosans and pentaclosans were

almost the same under the two photolysis experiments. Obviously, it was the initial triclosan/active chlorine ratio but not the initial concentrations of these two precursors that really influenced the production of chlorinated triclosans from triclosan under UV irradiation.

4. Conclusions

Chlorinated triclosan derivatives and dioxins, including the most toxic 2,3,7,8-TeCDD, were measured as the photo-transformation products of triclosan in the presence of active chlorine in seawater matrix under both UV irradiation and dark conditions. Both UV light and the initial concentrations of triclosan and active chlorine played important roles in the photo-transformation of triclosan to dioxins. Higher yield of dioxins was observed in the experiment with lower concentrations of triclosan and free chlorines and TrCDDs were found to be the most abundant dioxin products. On the other hand, the formation yields of chlorinated triclosans from triclosan was mainly influenced by the initial triclosan/active chlorine ratio, and UV light promotes the transformation of these compounds to dioxins, including the most toxic 2,3,7,8-TeCDD. Possible formation mechanisms of dioxins and chlorinated triclosan derivatives from the transformation of triclosan under both dark and UV irradiation conditions were proposed. Results from this study suggest that the transformation products of triclosan could be even more toxic and persistent in the environment than itself. More public awareness should be paid to the potential adverse human health effect resulting from the long-term exposure to triclosan and its transformation products in environmental waters.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jhazmat.2018.12.088>.

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